

## Appendix C

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(54) Ginseng-based composition for the treatment of an immune deficiency

(57) The present invention relates to a ginseng-based composition. The composition employs semi-wild red ginseng suspended in water or an aqueous product. Semi-wild red ginseng contains approximately 8% of saponins. Semi-wild red ginseng is suspended in an aqueous product or a pure powder in the proportion of 9.43g of powder in 100ml. The composition is given to be ingested. The invention is used, in particular, in the treatment of an immune deficiency.

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The present invention relates to a ginseng-based composition for treatment of an immune deficiency.

The actions of ginseng are known since ancient times. The medicine recommends the use of ginseng in the following cases: fatigue, depression, anemia, impotent, palpitations, diabates, chronic dyspnea, cardio-vascular trouble and arterial hypotension.

The present invention make it possible to struggle with immune deficiency and the cases mentioned above, by improving the self-defense system of organism.

The state of the art can be defined by the following patent: FR-A-2,531,864: The invention concerns an adminiculum which increases anti-tumor activity of mitomycine C and doxorubicine chloride.

This adminiculum include an extract as active component which is dissolved in water or aqueous-organic solvent. The active component is natural pharmaceutical product which consists of Astragali root, chinamon cortex, Rehmanniae root, Paeoniae root, Cnidii rhizome, Atractylodis lanceae rhizoma, Angelicae root, ginseng root, Hoelen and Glycyrrhizae root.

Application for treatment of tumor.

FR-A-2,532,179: The invention concerns a pharmaceutical preparation. This preparation includes an extract dissolved in water or aqueous organic solvent, as active components, such as several natural pharmaceutical product selected from the group consisting of Astragali root, Chinnamomi cortex, Rehmanniae root, Paeniae root, Cnidii rhizoma, Atractylodis lancea rhizoma, Angelicae root, ginseng root, Hoelen and Glycyrrchizae root.

This preparation remedies a symptom of cancer.

The present invention discloses, through a test of hemagglutination, a novel application of ginseng-based composition.

#### TEST OF HEMAGGLUTINATION

The results obtained by carrying out this test will permit to verify whether the ginseng can support the production of antibody in mouse C57BL subjected to the injection of sheep erythrocytes into the abdominal-cavity.

#### EXPERIMENTAL CONDITIONS

This experiment has been carried on the mouse of C57BL line breded by IFFA-CREDO.

Length of the experiment : 10 days

Date of begen to experiment : 17 Ougust 1987.

-Material

animals : four lots of 20 mouse C57BL which have mean weight between 18 and 23g are selected.

-Conditions of breeding

Temperature = 21C°

Humidity = 65%

Food = drinking and eating complete extra-labo,  
freely

OPERATION METHOD

Product to test : semi-wild red ginseng containing 8% of saponine.

The ginseng is suspended with water at a rate of 9.43g of powder in 100ml (suspension S). Thus, this solution contains 750ng of saponin for 100ml.

The lot A: female mice, which are numbered from A1 to A20, receive orally 0.2ml of the suspension per 10g of body weight.

The lot B: male mice, which are numbered from B1 to B20, receive orally 0.2ml of the suspension per 10g of body weight.

The lot C: female mice, which are numbered from C1 to C20, constitute the control lot.

The lot D: male mice, which are numbered from D1 to D20, constitute the control lot.

These animals are measured at time T = 0 and T = 8 days.

The animal behavior is observed daily during all the time of the experimentation.

In all the seven days, the mice receive 0.2ml of the suspension S per 10g of body weight by using stomach probe.

In parallel, the mice are subjected to the injection of sheep erythrocytes, which are suspended with physiological saline in the proportion of 3:5, into the abdominal-cavity. These injections are practiced for two days, in three times a day, in beginning at the time T = 0 and at a dose of 0.2ml/mouse.

Twenty four hours after the last administration of ginseng, the blood is collected by using a sharp pasteur pipet, on the level of sinus retro-orbital (between 1.5 and 5ml of blood per animal).

The collected serum is subjected to hemagglutination test according to the method of Takatsy, slightly modified as follows:

TREATMENT OF THE SERUM

Collect the blood in a clean and dry tube. Let the blood congregate slowly in the temperature of laboratory, decant the serum and centrifuge it. Don't use the milk-white or so much hemolyzed serum.

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The serum can be conserved at the temperature of 4 C° during four days.

#### Execution of the reaction

1) first dilution of the serum in a tube for hemolization. Apply 0.2ml of serum to experiment, and add 0.8ml of physiological saline. Let reposit during one hour at the ordinary temperature by agitating for fifteen minutes.

#### Titration of the agglutinin

Dispose twenty tubes for hemolization in 2 lines and 10 positions, and apply 0.25ml of physiological saline to all the tubes, except first tube.

Dispense the 0.25ml of experimental serum which is dissolved in 1/5, to the first tube and second tube.

Agitate the tube No.2, and then take 0.25ml and apply to tube No.3, agitate this tube and take 0.25ml which is applied to tube No.4, and so running until tube No.20.

Take 0.5ml and apply to tube No.21 which is conserved at 4 C° for making further dilution if necessary.

And then, add 0.1ml suspension of sheep erythrocyte dissolved into 2% in physiological saline. Agitate and leave it at the temperature of laboratory among two hours.

Agitate again, and at this time, make first observation. After eighteen to twenty hours at 4 C°, if necessary, make second observation by using a concave mirror.

Quotation of the result : 0,1,2

0 - no agglutination

1 - average agglutination

2 - full agglutination

#### RESULTS

The whole result is recorded in detail in the annexed table.

The limit of agglutination of serum lot A : 1/3494

The limit of agglutination of serum lot B : 1/3852

The limit of agglutination of serum lot C : 1/43

The limit of agglutination of serum lot D : 1/34

The average coefficient which determine the argumentation of antibody production in the animals treatmented with semi-wild red ginseng is as follows:

<u>Female control mouse</u>	=	<u>1/43</u>	=	81.255
Female treated mouse		1/3494		

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$$\begin{array}{lcl} \text{Male control mouse} & = & \frac{1}{34} = 113.294 \\ \text{Male treated mouse} & & \frac{1}{3852} \end{array}$$

$$\begin{array}{lcl} \text{Control mouse in total} & = & \frac{1}{38.5} = 95.558 \\ \text{Treated mouse in total} & & \frac{1}{3673} \end{array}$$

It is revealed that the coefficients are slightly different according to sex and the coefficients are significantly elevated in treated animals. It is confirmed that the activity of semi-wild red ginseng by antibody production.

#### CONCLUSION

The treatment of semi-wild red ginseng has exhibited significant improvement of antibody production.

The agglutination still shows antibody production activity up to the dilutions as follows:

Treated animals: male: 1/3852 female: 1/3494

Control animals: male: 1/34 female: 1/43

It is noted that the state of animal behavior remains normal, during all the times in the experiment, and the treated animals have slightly more growth than the control animals.

## Agglutination titer of serum lot A

N°	1/7 tube 1	1/14 tube 2	1/28 tube 3	1/56 Tube 4	1/112 tube 5	1/224 tube 6	1/448 tube 7	1/896 tube 8	1/1792 tube 9	1/3584 tube 10	1/7168 tube 11	1/14336 tube 12
A1	2	2	2	2	2	2	2	2	2	1	0	0
A2	2	2	2	2	2	2	2	2	2	0	0	0
A3	2	2	2	2	2	2	2	2	1	0	0	0
A4	2	2	2	2	2	2	2	1	0	0	0	0
A5	2	2	2	2	2	2	2	2	1	0	0	0
A6	2	2	2	2	2	2	2	1	0	0	0	0
A7	2	2	2	2	2	2	1	0	0	0	0	0
A8	2	2	2	2	2	2	2	2	2	2	2	0
A9	2	2	2	2	2	2	2	2	2	2	2	0
A10	2	2	2	2	2	2	1	0	0	0	0	0
A11	2	2	2	2	2	2	2	2	2	0	0	0
A12	2	2	2	2	2	2	2	2	2	1	0	0
A13	2	2	2	2	2	2	2	1	0	0	0	0
A14	2	2	2	2	2	2	2	2	1	0	0	0
A15	2	2	2	2	2	2	2	2	1	0	0	0
A16	2	2	2	2	2	2	1	1	0	0	0	0
A17	2	2	2	2	2	2	2	2	2	2	1	0
A18	2	2	2	2	2	2	2	1	0	0	0	0
A19	2	2	2	2	2	2	2	2	2	2	2	0
A20	2	2	2	2	2	2	2	2	2	2	1	0

Average dilution = 1/3496





Agglutination titer of Lot D

N°	1/7 tube 1	1/14 tube 2	1/28 tube 3	1/56 Tube 4	1/112 tube 5	1/224 tube 6	1/448 tube 7	1/896 tube 8	1/1792 tube 9	1/3584 tube 10	1/7168 tube 11	1/14336 tube 12
D1	2	2	2	1	0	0	0	0	0	0	0	0
D2	2	2	1	0	0	0	0	0	0	0	0	0
D3	2	2	1	0	0	0	0	0	0	0	0	0
D4	2	2	2	1	0	0	0	0	0	0	0	0
D5	2	2	0	0	0	0	0	0	0	0	0	0
D6	2	2	1	0	0	0	0	0	0	0	0	0
D7	2	2	1	0	0	0	0	0	0	0	0	0
D8	2	2	2	1	0	0	0	0	0	0	0	0
D9	2	2	1	0	0	0	0	0	0	0	0	0
D10	2	2	2	0	0	0	0	0	0	0	0	0
D11	2	1	0	0	0	0	0	0	0	0	0	0
D12	2	2	2	1	0	0	0	0	0	0	0	0
D13	2	1	0	0	0	0	0	0	0	0	0	0
D14	2	2	1	0	0	0	0	0	0	0	0	0
D15	2	2	1	0	0	0	0	0	0	0	0	0
D16	2	2	2	1	0	0	0	0	0	0	0	0
D17	2	2	2	1	0	0	0	0	0	0	0	0
D18	2	1	0	0	0	0	0	0	0	0	0	0
D19	2	1	0	0	0	0	0	0	0	0	0	0
D20	2	2	1	0	0	0	0	0	0	0	0	0

Average dilution = 1/34

Treated Lot A - weight (g) at time T = 0																			
A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20
19.1	0.4	18.3	19.8	19.5	0.1	18.0	18.6	19.4	18.9	0.5	18.7	18.7	0.5	18.5	19.5	19.1	19.0	19.0	0.0
Weight (g) at time T = 8 day																			
19.5	20.7	18.6	20.0	19.9	20.4	18.1	18.7	19.4	18.6	20.6	18.7	18.7	20.6	20.5	19.7	19.3	19.3	19.3	20.0
At time T = 0 : M = 19.3g (+1.27 ~ -1.23)																			
At time T = 8 : M = 19.5 (+1.1 ~ -1.4)																			

Treated Lot B - weight(g) at time T = 0																			
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	B18	B19	B20
19,5	20,2	20,4	21,3	20,9	19,9	19,8	21,8	22,1	20,7	20,8	19,8	19,5	19,7	20,8	21,6	20,5	19,5	21,6	20,2
Weight (g) at time T = 8 days																			
19,7	20,5	20,8	21,2	21,1	20,0	19,9	21,7	22,4	20,8	20,9	20,7	19,9	20,1	20,8	21,7	20,3	20,3	21,7	20,4
At time T = 0 : M = 20.52g (+1.875 ~ -0.825)										At time T = 8 : M = 20.745g (+1.655 ~ -1.045)									

Control Lot C - weight (g) at time T = 0																			
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
18.5	19.2	18.4	19.3	20.8	19.2	19.8	18.8	18.1	19.7	20.5	19.2	18.5	21.1	20.8	20.0	20.1	19.5	18.6	19.2
Weight (g) time T = 8 days																			
18.7	19.5	18.8	19.2	20.9	19.0	19.7	18.9	18.2	19.8	20.5	19.3	18.7	20.9	20.8	20.3	19.9	19.9	18.4	19.4
At time T = 0 : M = 19.465g (+1.335 -1.065)										At time T = 8 : M = 19.54g (+1.36 -1.14)									

Control Lot D - weight (g) at time T = 0																			
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20
20.5	21.2	20.4	22.5	21.8	19.8	19.8	20.9	21.1	21.7	20.5	20.4	19.5	21.1	20.5	20.2	20.8	19.2	19.3	19.1
Weight (g) at time T = 8 days																			
20.7	21.2	20.3	22.2	21.9	19.9	19.7	20.6	21.2	21.8	20.5	20.3	19.7	21.0	20.9	20.3	20.9	19.6	19.8	19.0
At time T = 0 : M = 20.51g (+1.79 - 1.41)      At time T = 8 : M = 20.565g (+1.635 - 1.565)																			

CLAIMS

1. Composition for treatment of an immune deficiency by improving the self-defense system of organism, characterized by that:

semi-wild red ginseng suspended in water, aqueous product or pure powder is utilized.

2. Composition according to the claim 1, wherein the semi-wild red ginseng contains about 8% of saponine.

3. Composition According to the claim 2, wherein the semi-wild red ginseng is suspended with aqueous product or pure powder in the rate of 9.43 g powder per 100ml.